Ozonated Liquids in Dental Practice – A Review.
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Part 8: Disinfection in Dental Practice.

Abstract: In Part 8 of Ozonated Liquids in Dental Practice, the uses of ozone are examined in the role of Disinfection in the Dental Practice. Some of these concepts can be taken out of the dental practice and placed into the at-home environment. There is an increasing body of research and evidence to support the use of ozone in both gas form and dissolved in water to control and eliminate infection and cross infection. Some of the information found in Part 5, Dental Unit Water Lines is found here, as this information forms the basis to change common practice and eliminate the potential cross-infective pathways. These may be created by compliance issues, or ignorance of modern and up to date cross infection controls. Splatter and aerosols from dental procedures may possibly infect health care personnel (*Wirthlin et al 2003*). The safety of dental waterlines has been questioned on national TV in the USA (*PubMed 2000*). Szymanska (*Szymanska 2005*) identified moulds, bacteria and yeasts in biofilms. Some of these in certain circumstances, especially in people with immunological disorders, may be a cause of opportunistic infections (*Szymanska 2005*).

The use of ozonated liquids in operative dental care is supported by the published research. There is a wealth of research and information to show that the integration of ozone into clinical dental care reduces the need to amputate dental structures and tooth tissue. Ozone has been shown to arrest and reverse decay, to eliminate infection and control wound healing. It reduces and can eliminate volatile sulphur compounds, and bio-molecules found in caries, plaque and as a general oral loading. Ozone eliminates bio-films in dental unit tubes, and when used as part of a surgical protocol, enhances tissue healing and establishes health faster than control subjects. Published research suggests that ozone has an important role to play in clinical patient care by reducing fear and anxiety.

Introduction.

The quality of dental unit water is of great importance since patients and dental staff are regularly exposed to water from aerosols generated during work, and Dental Unit Water Line (DUWL) contamination has been shown to be a concern (*Putnins et al 2001, Wirthlin et al 2003*). Biofilms are a natural occurrence in aquatic environments, including community drinking water systems.

The interior of small-diameter tubing in dental unit waterlines are also sites of biofilm formation. The physics of fluid flow dynamics result is a static outer liquid layer covering the inner aspect of the tube or pipe.

Water drawn from the main supply contains a staggering quantity of micro-organisms per ml which is allowable by various laws enacted in each country. These water-born micro-organisms have the potential to cause infection and disease.

For the vast majority of the population, this does not occur due to the concept of 'herd-immunity' – a term used to describe the majority of a population group who are immune or have resistance to a disease or infectious organism. For a small minority of the population, this is a concern, due to pre-existing health issues and pharmacological agents that can reduce the viability of the individual's immune system and their ability to resist infection.

In the case of the dental unit, water becomes stagnant when not in use. Molecules precipitate from the water onto the interior wall and promote the adherence of planktonic micro-organisms from the water. Once they become sessile (ie, static and fixed in place), the micro-organisms change their phenotype. After adherence, there is a so-called surface-associated lag time, and the organisms then enter a growth phase and produce exopolysaccharides that coat the organisms in a slime layer. This is the start of the biofilm formation.

Within this biofilm, the micro-organisms can signal one another, transfer nutrients, and exchange genetic material. The insoluble exopolysaccharides shield the micro-organisms from displacement and from penetration by predator organisms, antibiotics, and disinfectants. In many ways, this biofilm is no different to that which forms in the oral cavity and is called plaque. The external surface layer of micro-organisms is faster growing and may detach as "swarmer" cells. Detachment of micro-organisms from dental unit biofilm flushed into the oral cavity could theoretically infect the patient.

Splatter and aerosols from dental procedures may possibly infect health care personnel (*Wirthlin et al 2003*). DUWL contamination has become a concern to clinical dentistry (*Putnins et al 2001*).In one study, a viability staining technique identified significantly more bacteria in water than could be cultured (*Putnins et al 2001*). The mean LPS levels in water collected from high-speed and air & water spray lines in use were 480 and 1,008 endotoxin units (EU)/ml. This was significantly higher than the mean level of 66 EU/ml found in water samples collected from adjacent clinic sinks (*Putnins et al 2001*). In order to satisfy water regulations and comply with health and safety legislation dentists should institute infection- control measures to maintain the dental unit water at the standard of less than 200 colony-forming units per ml of aerobic bacteria (*Pankhurst 2003*). However, this may be inadequate with groups of immuno-compromised patients.

Bacteria have been around for millions of years, and are not without a trick or two of their own when survival is threatened. The vast majority of anti-microbial products act over a period of time. This window of opportunity is used by micro-organisms to evolve new species, termed 'resistance', to these disinfection products.

Modern health care now faces the problem of bacterial strains which are resistant to a wide variety of products. In a world where the life expectancy has been lengthened by pharmaceuticals, micro-organisms are now faced with the ultimate choice of host. The micro-organisms' host is beset with immunological conditions that lower the innate immune system's ability to contain and

repel infection. It is an era of opportunistic infection, and as their hosts tend to live in crowded surroundings, conditions are perfect for micro-organism evolution, cross-infection and survival.

DUWL's are ideal environments for the growth of micro-organisms entering dental units from the municipal water supply (*Barbeau 2000*) and from previously treated patients (*Montebugnoli et al, 2004*). Very few cases of cross-infection have been linked directly to contamination in DUWL's, but in an era of sociological changes, this risk has grown proportionally (*Szymanska 2005*). Al Shorman *et al* (*Al Shorman et al 2003*) discussed that Pseudomonas aeruginosa found in DUWL's in Belfast Dental School could be a risk for immuno-compromised adults and cystic fibrosis children for example.

Microbiological Studies of DUWL.

A Jordanian study (*Al-Hiyasat et al 2007*) illustrated that stasis in DUWL's during non-working time results in the proliferation of the biofilm and colony forming units (CFU's). Overall, the highest counts (log (10) count CFU ml (-1)) were found at the beginning of the working day (1.38 +/- 1.05), and the lowest counts after flushing for 2 min (1.10 +/- 1.03). An increase in the number of CFU's were seen again at midday (1.15 +/- 1.04) ($P < 0.05$).

Various studies have looked at DUWL's to categorise the microbiological flora involved in the formation of biofilms. Szymanska (*Szymanska 2005*) identified moulds: Aspergillus amstelodami, Aspergillus fumigatus, Aspergillus spp. from Aspergillus glaucus group, Aspergillus repens, Citromyces spp., Geotrichum candidum, Penicillium aspergilliforme, Penicillium pusillum, Penicillium turolense, Sclerotium sclerotiorum: yeast-like fungi: Candida albicans, Candida curvata and other yeasts in a Polish study. Some of them, in certain circumstances and especially in people with immunological disorders, may be a cause of opportunistic infections.

In Ireland, Al Shorman *et al* (*Al Shorman et al 2003*) and in Jordan Al-Hiyasat *et al*, (*Al-Hiyasat et al, 2007*) evaluated the extent of Pseudomonas aeruginosa contamination of DUWL's at Dental Teaching Centres. Dental units from clinics in conservative dentistry, periodontology, and prosthodontics were examined in the Jordanian study. Al-Hiyasat *et al* detected P. aeruginosa in 86.7% (26/30) of the dental units at the beginning of the working day, and in 73.3% (22/30) after 2 min of flushing and at midday. Conservative dentistry units had the highest counts, followed by periodontology and prosthodontics ($P < 0.05$). Al Shorman et al (*Al Shorman et al 2003*) showed a reduction in the total volume count (TVC) of water from the control unit from 2.3 x 104 (week 1) and 3.4 x 104 CFU/mL after 2 weeks of installation. The primary coloniser was identified (API 20 NE kit) as pure P aeruginosa.

O'Donnell *et al* (*O'Donnell et al 2006*) found the most common bacterial species cultured from the mains water were Micrococcus luteus and Sphingomonas spp., respectively, the latter of which are known opportunistic pathogens. Montebugnoli *et al* in their 2004 paper (*Montebugnoli et al 2004*) discussed direct person-to-person transmission of periodontal bacteria through saliva. Dental units have been demonstrated to retract saliva from patients under treatment and to release it into the mouths of subjects undergoing the next operation.
A polymerase chain reaction-based method was used to investigate periodontal pathogenic bacteria inside DUWL's. The presence of DNA of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Treponema denticola was examined, and positive samples of Prevotella intermedia DNA found. These findings clearly suggest that dental units have the potential to transmit periodontal pathogens between patients.

Porteous *et al* (*Porteous et al 2004*) found non-tuberculosis mycobacteria in DUWL at a hospital dentistry clinic where immuno-compromised patients are seen. Mycobacterium simiae was isolated from one of the four pre-treatment samples and from two of the four post-treatment samples. Mycobacterium mucogenicum was isolated from one of the four post-treatment samples.

Microbiological Studies of Turbine and 3-in-1 Spray.

A study in 2005 by Szymanska (*Szymanska 2005*) examined bacterial endotoxin concentration in the water flowing from a high-speed hand piece of a dental unit and in the air contained in the bio-aerosol formed during dental conservative treatment. The air was collected in the space between the patient and dentist. The study was conducted on 25 operative units and had two stages: before application of a DUWL disinfectant and after a 2-week application of disinfection procedure.
The research showed that the mean concentration of bacterial endotoxin in the water flowing from high-speed hand pieces was significantly reduced after the use of a disinfectant. The mean concentration of bacterial endotoxin in the air was similar at both stages - before and after application of waterline decontamination procedure.

The study showed that in dental air-water aerosol, water is the main source of bacterial endotoxin contaminating the aerosol during the work with dental hand pieces.

In Japan, Kohno *et al* (*Kohno et al 2004*) found that the mean viable bacteria count was 910 -/+ 190 CFU/ml at the end of dental hand pieces, and 521 -/+ 116 CFU/ml at the end of three-way syringes.

Contamination of DUWL with Cleaning Fluid Residue and Resin Bonding Issues.

In response to concerns of bacterial biofilm colonization of DUWL, a wide range of commercial intermittent and continuous chemical treatments for DUWL have been developed and marketed. Roberts *et al* (*Roberts et al 2000*) researched the possible effect of continuous chemical treatment regimens on dentin-bonding agents. Four proposed antimicrobial agents for use in DUWL on dentin bond strength were examined. The authors used a fifth-generation dentin-bonding agent to bond composite cylinders to molar dentin surfaces. They then used selected antimicrobial agents as rinsing agents after conditioning.

The composite cylinders were shear tested, and their fracture strengths were compared statistically. All proposed antimicrobial agents reduced dentin bond strength. Proposed waterline treatment regimens of a diluted mouth rinse and chlorhexidine significantly reduced dentin bond strength compared with sodium hypochlorite and citric acid regimens. The clinical implications of this 2000 research were that DUWL antimicrobial agents may adversely affect dentin bonding strength.

A 2001 study showed there was no significant difference in shear bond strengths of resin-based composite to tooth structure when rinsed with distilled water mixed with mouthwash, distilled water or water from a municipal source (*Knight et al 2001*). The clinical implication is that DUWL disinfected using a diluted mouthwash solution may be used while bonding resin-based composite to either enamel or dentin. However dental mouth wash is not a particularly good sterilizing medium.

Another 2001 study suggested that DUWL biocides may adversely affect adhesion of resin to enamel (*Taylor-Hardy et al 2001*). This study evaluated the effects of chemical biocides used to

control dental unit waterline biofilm on the bond strength of resin to enamel. Sixty bovine teeth were randomly assigned to six treatment groups. One-way ANOVA revealed a significant difference in means ($p < 0.001$) and Tukey's multiple range test indicated that three of the experimental groups had significantly lower mean shear bond strengths than the control ($p < 0.05$).

A 2004 study examined the effects of biocide contamination of DUWL (*von Fraunhofer et al 2004*). In their closing discussion, they comment on the varying reports on the effects of such agents on the bond strength of restorative dental materials and, particularly, between these agents and dental hard tissues.

Failure of the enamel-resin bond can lead to marginal micro-leakage around the periphery of the restoration. Failure of this margins results in staining – the ingress of diet-related chromogenic organic molecules, establishment of the acid-niche environment, and eventual failure of the restorative care.

The outstanding results of the Al Shorman *et al* (*Al Shorman et al 2003*) paper, where the bacterial count of samples collected showed a bacterial reduction from 5.2*103 CFU/ml before treatment to 300 CFU/ml after the first O_3 application and then to 0 CFU/ml after the second application onwards, points to the use of ozone as the DUWL sterilisation method of choice and state-of-the-art. Con-current studies have shown that ozone does not interfere with dental material bond strengths or material retention (*Campbell et al 2003* and *Abu-Naba'a et al 2004*).

The findings from the Campbell *et al* (*Campbell et al 2003*) study were illustrated in the Holmes study (*Holmes 2004*) where these bonding issues were further examined. After ozone treatment, resin bonding was carried out over *soft, previously-infected dentine*. This flies in the face to all conventional teaching with regards to cavity preparation and dentine bonding protocols. The results from the Holmes study at 6-months showed that ozone treatment returned *alpha* scores on the UHPS criteria for all restorations placed in this way. It is argued that the incorporation of ozone into a dental unit will have a major impact on the standard of care delivered by a dentist with either limited investment in instrumentation, or those of mediocre skills.

Implications in Dental Surgery Wound Healing.

In the Putnins *et al* (*Putnins et al 2001*) paper in 2001, the role of the infective biofilm in DUWL's was discussed with relation to surgery. As it was not possible to reduce the CFU count to zero, the authors concluded that the presence of high heterotrophic bacterial counts, sloughing biofilm, and high LPS levels posed a real risk to periodontal wound healing biology. This can be widened to include ***any*** form of dental surgery from tooth removal, to implant placement. Most dental practices in the western world use sterile saline, but it other areas of the world this is not available for routine use. The incorporation of ozone would of course revolutionise not only the pre- and post- surgical aspects, but the surgical process itself. Ozone is known to encourage wound healing as well as control opportunistic infection (*Bocci 1994*).

Solutions to the Infective Biofilm.

In a study from the United States of America in 1997 (*Murdoch-Kinch et al 1997*) the effectiveness of American Dental Association (ADA) recommended approaches for reducing DUWL contamination were investigated using newly installed dental units. Over a 2-month period, the authors studied independent water reservoirs, a sodium hypochlorite disinfection regimen, daily draining and purging of DUWL's and point-of-use filters by assessing microbial

contamination and biofilm development using scanning electron microscopy. The findings demonstrate that DUWL contamination can be controlled when dental personnel use available technologies and adhere to recommended maintenance protocols. However, employee compliance with instructions is an issue, in the same way the dental profession whinge about patient compliance to oral hygiene instructions and dietary advice.

In Jordan, Al-Hiyasat *et al*, (*Al-Hiyasat et al 2007*) found that flushing the dental unit for 2 minutes significantly reduced the counts of P. aeruginosa, but flushing with infected water is not going to eliminate the biofilm, nor will it reduce the CFU count to zero.

Another study from the USA in 2002 (*Cobb et al 2002*) concluded that after four minutes of continuous flushing (the current ADA recommendation), all waterlines still harboured CFU levels that exceed current ADA recommendations. Cobb *et al* concluded that water flushing of DUWL's produced a statistically significant reduction in planktonic bacteria at each time interval compared to the baseline and between each successive time interval. However, the level of CFU's after four minutes of continuous water flushing still exceeded the current ADA recommendations for acceptable levels of micro-organisms.

Wirthlin *et al* (*Wirthlin et al 2003*) in their 2003 paper showed that chlorine dioxide waterline cleaners are effective in decontaminating DUWL biofilms. Chlorine dioxide has advantages over other chlorine products: it does not form carcinogenic compounds, has a long shelf-life in comparison with other products, and is not a strong irritant. These authors concluded that controlling DUWL biofilm would have beneficial effects on nosocomial infections.

A study published by Nagayoshi, Fukuizumi *et al* (*Nagayoshi, Fukuizumi et al 2004*) examined the effect of ozonated water on oral micro-organisms and dental plaque. Almost no micro-organisms were detected after being treated with ozonated water (4 mg/l) for 10 s. When the experimental dental plaque was exposed to ozonated water, the number of viable S. mutans remarkably decreased. These researchers noted that ozonated water strongly inhibited the accumulation of experimental dental plaque in vitro. After the dental plaque samples from human subjects were exposed to ozonated water in vitro, almost no viable bacterial cells were detected. These results suggest that ozonated water should be useful in reducing the infections caused by oral micro-organisms in dental plaque.

A further study by Nagayoshi, Kitamura, *et al* (*Nagayoshi, Kitamura, et al 2004*) examined the effect of ozonated water against Enterococcus faecalis and Streptococcus mutans infections in vitro in bovine dentin. After irrigation with ozonated water, the viability of E. faecalis and S. mutans invading dentinal tubules significantly decreased. These researchers concluded that used with ultrasonic instrumentation, ozonated water application may be useful for endodontic therapy.

Kohno *et al* in 2004 (*Kohno et al 2004*) published their results that indicated acidic electrolyzed water could be applied as an appropriate measure against bacterial contamination of the DUWL. Montebugnoli L *et al* concluded in their 2004 paper that dental manufacturers should be invited to design dental units that incorporate automated devices to disinfect DUWL's between patients with minimal effort by dental staff (*Montebugnoli et al 2004*). Porteous *et al* urged dental practitioners in 2004 (*Porteous et al 2004*) to understand the limitations of available DUWL treatments, and to consider the use of sterile water for non-surgical, as well as surgical, treatment of immuno-compromised patients to reduce the risk of cross infection.

In 2005, Szymanska (*Szymanska 2005*) concluded that the application of a disinfection product containing hydrogen peroxide caused a significant decrease both in the number of total fungi and

individual fungal species. This confirmed his assertion that hydrogen peroxide was effective for fungal decontamination of DUWL's. In another paper from 2005 titled '*Microbiological Studies of Turbine Spray*', Szymanska commented that the application of a user-friendly water disinfectant to significantly decrease endotoxin concentration in the aerosol is one of recommended methods to reduce health risk.

O'Donnell *et al* (*O'Donnell et al 2006*) discussed a Water Management System, described as 'an integrated and automated DUW cleaning system'. This was investigated over a 12-month period. The system uses hydrogen peroxide- and silver ion-containing disinfectants in a once-weekly disinfection protocol.

Ozone has been used for purification of water due to its efficiency and lack of side effects. It has been used in the medical profession since the late 19th Century to treat infections and aid wound healing. In the 1920's Dr Edwin Parr, a Swiss dentist started, to use O_3 as part of his disinfection system. The use of O_3 mushroomed until the inter-war periods, when the advent of cheap chlorine saw the use of O_3 decline. The pharmaceutical industry began to flood the market with the wide variety of anti-microbials we know today.

The vast majority of anti-microbial products act to kill micro-organisms over a period of time. This window of opportunity is used by bacteria to evolve resistance to these disinfection products, and modern health care now faces the problem of bacterial strains which are multiple-product resistant. The micro-organisms' host is beset with immunological conditions that lower the innate immune system's ability to contain and repel infection. And there is a trend to increased life span that requires pharmaceutical products for continued survival. The risk of cross-infection into this group of the population cannot be over looked.

Looked at in terms of: '*What is the perfect anti-microbial agent?*' ozone would seem to fit the required profile. Ozone acts instantly, by oxidising bacteria, fungi, viruses, prions, and their effluent bio-molecules. Micro-organisms cannot evolve fast enough to develop resistance to O_3, so it remains the 'perfect' disinfection and sterilisation product to use.

However, O_3 is not without its own issues. From a physical property perspective, O_3 is a very unstable gas, and has to be manufactured at the point of use. The equipment to deliver O_3 has an associated cost. But it is a one-time investment that is still more economical than disinfectant use.

Ozone leaves no biocidal traces so the risk of contamination in bonding procedures is removed. The potential health risk with free O_3 in the oral cavity and the work place must be addressed, and this would be carried out as part of the risk assessment and design of the ozone system integrated into the dental unit.

In an early paper from 2002 (*Cardon et al 2002*) Cardon BE *et al* concluded that an ozonation system evaluated appeared to have no long-term benefit on DUWL biofilm control. However on closer reading, the concentration of O_3 used, 0.01 to 0.06 ppm, would not have been sufficient to lower high CFU levels or eliminate the DUWL biofilm. Not surprising, CFU values after O_3 treatment of excess 10,000 CFU were reported.

Al Shorman *et al* (*Al Shorman et al 2002*) used O_3 at a concentration of 2100 ppm bubbled into 1 litre of water over a 10-minute time period. O_3 formed from dry air resulted in a bacterial reduction from 5.2*103 CFU/ml before treatment to 300 CFU/ml after the first O_3 application and then to 0 CFU/ml after the second application onwards. The authors commented on how low the concentration could be lowered and retain efficacy. Puttaiah and Lin (*Puttaiah and Lin 2006*) in

an IADR abstract published in 2006 used 0.8 ppm of ozonated water as irrigant. At the end of week four all Units showed counts > 500 cfu/mL. They concluded that an initial cleaning with 60 ppm ClO2 and use of 0.8 ppm O_3 mixed in water as irrigant controlled contamination up to 30 days.

In a follow-up study in 2003, Al Shorman *et al* (*Al Shorman et al 2003*) compared hydrogen peroxide and O_3 DUWL decontamination. Hydrogen peroxide continuously produced water with a Total Viability Count (TVC) of less than 100 CFU/mL. The TVC of water from the control unit was 2.3 x 104 and 3.4 x 104 CFU/mL after 1 and 2 weeks of installation. After the first O_3 treatment the TVC was reduced to 60 CFU/mL and rose to 3.9 x 104 CFU/mL after a week with few Pseudomonas colonies. After two weeks, TVC was 2.8 x 103CFU/mL CFU/mL and became 0 CFU/mL after the treatment. Repeated sampling of the unit for 9 weeks showed 0 CFU/mL. Flushing with water could not maintain a CFU or TVC value within acceptable potable water standards (200 CFU).

In 2007, Shenberg *et al* (*Shenberg et al 2007*) showed ozone is extremely reactive towards selected carious dentine biomolecules, and such reactions are likely to be of relevance to its reported microbiocidal activity. High resolution proton (1H) nuclear magnetic resonance (NMR) spectroscopy was used to determine the nature and extent of the oxidation of biomolecules present in carious dentine, plaque and saliva. Experimental samples were treated with ozonated (2 ppm) water. The Shenberg *et al* results mirrored previous studies (*Holmes 2003* and *Holmes 2003*) where ozonated water showed marked reductions in volatile sulphur compounds. In these earlier studies, the Halimeter, a volatile sulphur detection system, was used. In the Shenberg 1H NMR study, ozone dissolved in water was shown to attack:

- a-D-glucose, giving rise to formate as it's by-product
- pyruvate with acetate and CO2 via an oxidative decarboxylation process
- amino acid volatile sulphur compound precursors cysteine and methionine were oxidatively transformed to their corresponding primary oxidation products, cystine and methionine sulphoxide respectively

These results are similar to the published 1H NMR studies from previous years where carious tissue samples were treated with ozone. The Shenberg study shows that ozone dissolved in water has the ability to denature bio-molecules seen in active decay and also found in oral saliva.

The Use of Ozone to Sterilise and Clean Air Supplies.

Ozone has been used to clean and sanitise air for over 100 years. The first reports of a public system come from the London Underground in the United Kingdom. Here, the subterranean portions of the railway network were supplied with fresh air that had been ozone treated (*Betjeman 1972*). It is known that usage of ozone-oxygen mixture is effective to eliminate pyogenic flora, tuberculosis agent, diphtheria and gas gangrene (*Apsatarov 1994, Vasil'ev & Markov 1992*) for example. The high performance of an ozone-oxygen mixture and its effect on Mycobacteria Tuberculosis has been published in the late 1990's (*Priymak et al 1991, Belyanin et al 1997*).

One published study (Fig 8.01) from the USA illustrated how ozone-treated air could control cross-infection amongst school children and their teachers. Published in 'Report to National Warm Air, Heating and Ventilating Association', James Steward, MD, the Director of Hygiene &

E. S. Hallett, Chief Engineer, Board of Education, St. Louis examined the effect of ozone to control cross infection in air-born diseases.

During the influenza epidemic in St. Louis, the most critical and advanced cases were transferred to an open air school, which made for high percentage of mortality. In one particular ward, experiments were made with ozonized air on cases approaching or at the crises period of the diseases where patients were able to inhale at all, they were at once relieved and successfully carried through the crisis point.

Two schools were then used for an experiment, one with ozonated air and another with ordinary air. Both schools contained approximately the same number of rooms. The following cases of sickness were observed and tabulated:

Infection Type	Ozone-Treated Air	Untreated Air
Tonsillitis	13	57
Sore Throat	24	60
Colds	46	64
Headache	9	66
Stomach ache	0	25
Earache	1	15
Toothache	0	20
Indigestion	0	9
Fever	1	49
The Grippe	0	6
Pneumonia	0	4

Table 8.01: Infection Control & Air Sanitisation with Ozone

Comparing the total days absent they found that in the school where ozonated air was used, the school children were absent, due to the foregoing cases of sickness, 475 school days, while in the school where ordinary air was circulated by means of the ventilating system, the school children were absent a total of 1,098 school days.

Steward and Hallett concluded; ‘Thousands of lives would be saved every year if homes and schools were equipped with apparatus for the circulation of ozone. Injected with the air of the building to the extent of one part of ozone to one million parts of air, it effects approximately 100% purification. In five years that ozone has been used in the Public Schools of St. Louis, tuberculosis cases have been reduced by 50%, and in addition, other diseases have been materially reduced.’

The Use of Ozone for Air and Surface Sterilisation.

Ozone is a powerful germicidal as was first indicated by Frohlick. Its high germicidal activity is has been shown to be due to its oxidizing power. Ozone is extensively used for the sterilization of public water supplies, for the treatment of wounds in hospitals, and for various purposes of sterilization and preservation in agricultural industries.

Some sterilization is effected by ozonation of air, since a marked reduction is obtained in the bacterial count of the air which has actually passed through the ozone generator and subsequent action of the generated ozone on the ambient surrounding air.

Ozone Concentration mg./1 hr	Time of ozonation Minutes	Bacterial count after 36 hrs. incubation	Mortification Percent colonies
174.3	0	Ca 2-3000	00
174.3	2	60	98
174.3	8	15	99.5

Table 8.02: Action of Ozone on Surface Cultures - E. coli

Ozone Concentration mg./1 hr	Time of ozonation Minutes	Bacterial count after 36 hrs. incubation	Mortification Percent colonies
174.3	0	1126	00
174.3	2	0	98.1
174.3	8	0	100

Table 8.03: Action of Ozone on Surface Cultures - Bacilli Diphtheria

Ozone Concentration mg./1 hr	Time of ozonation Minutes	Bacterial count after 36 hrs. incubation	Mortification Percent colonies
174.3	0	840	98
174.3	2	0	100

Table 8.04: Action of Ozone on Surface Cultures, Staphylococcus

Ozone Concentration mg./1 hr	Time of ozonation Minutes	Bacterial count after 36 hrs .incubation	Mortification Percent colonies
174.3	0	Ca 2000	0
174.3	2	Sterile	100

Table 8.05: Action of Ozone on Surface Cultures- Streptococcus

These tables illustrate the oxidative power of ozone on bacterial colonies grown macroscopically and dried on a soil medium.
The first table shows a bacterial kill rate of 98% and 99% of the bacterium E.. coli within two minutes and this confirms completely the results of Dr. Heise. It also gave evidence that the action of ozone is very intense on tall bacterial colonies six hours after inoculation.

In this study, other plates inoculated with dysentery, streptococcus, staphylococcus, ozonated 3-4 hours after vaccination were sterile alter two minutes.

The author concluded that '*According to the results of these experiments as shown in these tables, the disinfectory germical action of Ozone must be considered as most excellent and superior to other methods*'.

There are a number of industrial ozone units that produce a fine 'fog' of ozonated water. This is an ideal medium to sterilise a small to large room volume. Units could be developed for the dental practice market. Residual water vapour may be an issue in some settings, and each area would have to be assessed on an individual basis.

A study in 2002 showed that ozone dissolved in water prevents the spread of infective spores. Young and Setlow (*Young and Setlow 2004*) determined that ozone does not kill spores by DNA damage. Rather, ozone seems to render the spores defective in germination, perhaps because of damage to the spore's inner membrane. Spores are exceptionally resistant to cleaning and sterilisation methods. This paper illustrated the power of ozone to eliminate the most resistant infective bodies.

In South Africa, a study is underway to examine the use of a small ozone generator to sterilise and de-odorise animal kennels. Each kennel area is supplied with a small generator that can be activated once the kennel area has be vacated. Preliminary studies are very promising. This type of application can be taken out of the animal setting and redesigned for the dental and medical practice environment. Ozone is used extensively in the hospitality market to remove unwanted odours of animals and cigarette smoke. Small portable generators are used from room to room, and have been show to be very effective.

A Suggested Solution to the Issue of Cross Infection Control.

The use of ozone as the method of disinfection would offer the best solution as part of an integrated approach to dental care. Indeed, O_3 would seem to offer the opportunity of unit sterilisation which is a very different approach to dental unit care. Ozonated water exceeds all current standards for water quality in DUWL's at high enough concentrations.

Many dental units incorporate a bottled water delivery system, where filtered, purified and sterile water can be fed direct into the dental unit. If this water were to be further ozone treated on a continuous process, the formation of the biofilm would be eliminated, and issues of cross infection of a population of immunocompromised patients addressed.

The studies that have looked at air sterilisation suggest that air-born infections can be dramatically reduced and eliminated by ozone treating air supplies, either directly into the air-conditioning system, or as self-contained ozone generators in discrete room volumes. The use of ozone in the USA for air sterilisation is subject to debate, as certain US States consider ozone to

be a pollutant and a dangerous gas. This suggests a great deal of education needs to be carried out before the dental and medical profession can move forward.

As a final word of caution, the effect of sterile air on the herd immunity should be commented on. In today's so-called modern society, the incidence of asthma and bronchial-related illness and disease is on the increase.

It is not uncommon, especially in the USA for a family unit to live in an environmentally controlled home unit; to move through a closed environment into the garage and into a vehicle, that contains another environmentally controlled environment and then to drive and exit into the shopping mall, restaurant or office, where the same environmentally controlled surrounding and air are created. The potential for infection is reduced to near zero. But so to is the development of immunity for this and thousands of similar family units, so that the overall herd immunity reduces to a point where viruses and respiratory infections become serious life-threatening infections, not just a sniffley runny nose. The old practice of the mumps or measles party has been abandoned in favour of isolation, and avoidance of the infection. At some point in time, 'healthy' infections needs to be addressed and accepted back into the community.

Units Available for Water Sterilisation from Lime Technologies Holdings Limited.

There are many papers that suggest the frequent usage of ozonated water will improve oral hygiene, health and has many other uses, from sterilization of tooth brushes, dentures to surface sterilization such as wounds and suture lines, and disinfection of work surfaces.

Fig 8.01 The LT-WSU3 Unit from Lime Technologies Holdings Ltd

The LT-WSU3 is supplied with a 110-240 V AC power adaptor that supplies 12V DC to the unit. Fuse protection and an internal one-way valve add protection to the unit against water ingress by siphoning.

The LT-WSU3 unit is manufactured by Lime Technologies Holding Limited and distributed through their network of agents and distributors. It costs 280 Euros.

Ambient air is compressed and taken through a drier unit that typically gives a year's working time in humid conditions. From the drier unit, air is taken through a 1gm ozone generator built to Lime Technologies specifications. Operation is very simple, and power can be from the included mains power supply, or as optional extras, a car 12V DC connection or a solar array for use in remote areas.

The ability to use this unit away from mains supply makes the LT-WSU3 unit the unit of choice to prevent water born infections in remote areas and when camping. The auto-sensing power

supply allows the owner to make pure sterile water when on holiday in any location where water quality is questionable.

An alternative system is the TherOzone Unit, manufactured in the USA. This is based on the Soda Stream principle, where ozone is injected under pressure into a 1 litre bottle. At 3900.00 US $ it represents a sizable investment. This is similar to a design by O3 in South Africa. Ozone injection systems in a closed system such as these have a short ozone-water contact time. A concern is that these may be too short to give reasonable ozone levels in water.

Units Available for Air Sterilisation from Lime Technologies Holdings Limited.

Fig 8.02. The LT-RAS3 from Lime Technologies Holdings Ltd

The LT-RAS3 unit is a self-contained air steriliser unit. The LT-RAS3 can be wall mounted, placed on a table or shelf top.

It is supplied complete with an auto-sensing 110-240 VAC Mains Power Supply Unit that supplies 12 VDC to the unit.

Two timers make the setting up of the LT-RAS3 simple. One timer controls the time on, and the second, the time off. Periodic servicing by the customer is required to keep the ozone generator free from dust and debris build-up.

It costs 275.00 Euros direct from Lime Technologies Limited, www.limetechnologies.net

Conclusion.

Ozone has been shown not to interfere with dental material bond strengths, and there is evidence that it may increase material retention (*Abu-Naba'a et al 2004, Campbell et al 2003*).

Bocci (*Bocci 1994*) has emphasised that the potential toxicity of O_3 should not preclude its employment for medical, dental & veterinary purposes. This statement has been echoed by thousands of health professionals who use ozone in clinical practices around the world, and millions of patients that have been treated. The results of these studies show that ozone reduces the necessity for filling materials of unknown long-term potential toxicity.

The use of ozone as the method of disinfection would offer the best solution as part of an integrated approach to dental care. Indeed, O_3 would seem to offer the opportunity of unit sterilisation which is a very different approach to dental unit care. Ozonated water exceeds all current standards for water quality in DUWL's at high enough concentrations.

Gaseous ozone has long been observed to remove unwanted odours from air and fabrics, without damaging fabrics, for example, in home and hospitality settings. The paper by Destaillats *et al* in 2006 (*Destaillats et al 2006*) examined the chemical pathways of how ozone removes nicotine desorption from surfaces.

As part of a dental treatment unit, ozone can easily be integrated into routine dental care. This aspect of dental and health care has been reported in previous papers by Holmes J Lynch E and Filippi A.

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